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Changes in Labile Organic Carbon Fractions and Soil Enzyme Activities after Marshland Reclamation and Restoration in the Sanjiang Plain in Northeast China

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Abstract The extensive reclamation of marshland into cropland has tremendously impacted the ecological environment of the Sanjiang Plain in northeast China. To understand the impacts of marshland reclamation and restoration on soil properties, we investigated the labile organic carbon fractions and the soil enzyme activities in an undisturbed marshland, a cultivated marshland and three marshlands that had been restored for 3, 6 and 12 years. Soil samples collected from the different management systems at a depth of 0–20 cm in July 2009 were analyzed for soil organic carbon (SOC), dissolved organic carbon (DOC), microbial biomass carbon (MBC) and easily degradable organic carbon. In addition, the activities of the invertase, β-glucosidase, urease and acid phosphatase were determined. These enzymes are involved in C, N and P cycling, respectively. Long-term cultivation resulted in decreased SOC, DOC, MBC, microbial quotient and C (invertase, β-glucosidase) and N-transforming (urease) enzyme activities compared with undisturbed marshland. After marshland restoration, the MBC and DOC concentrations and the soil invertase, β-glucosidase and urease activities increased. Soil DOC and MBC concentrations are probably the main factors responsible for the different invertase, β-glucosidase and urease activities. In addition, marshland restoration caused a significant increase in the microbial quotient, which reflects enhanced efficiency of organic substrate use by microbial biomass. Our observations demonstrated that soil quality recovered following marshland restoration. DOC, MBC and invertase, β-glucosidase and urease activities were sensitive for discriminating soil ecosystems under the different types of land use. Thus, these parameters should be considered to be indicators for detecting changes in soil quality and environmental impacts in marshlands.

Keywords Cultivated marshland · Restored marshland · Soil enzyme · Microbial biomass carbon · Dissolved organic carbon · Easily degradable organic carbon

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Introduction

One of the major land use changes in northeast China in recent decades has been the reclamation of marshland. For example, the natural marshland area in the Sanjiang Plain decreased from 353×10^4 ha in 1954 to 81×10^4 ha in 2005 (Huang and others 2009). Previous studies suggested that extensive reclamation of marshland into cropland has had tremendous effects on soil properties, which has resulted in a significant increase in the oxidation of soil and the mineralization of soil C and N and has induced significant changes in soil biological properties (Raiesi 2006; Zhang and others 2007a). Zhang and others (2007a) reported that the land use/cover change in the Sanjiang Plain had resulted in a significant decline in soil microbial biomass carbon (MBC). In addition, the abandonment of cultivated marshlands resulted in increased dissolved organic carbon (DOC) and MBC (Zhang and others 2007b). Investigating the changes in soil labile organic carbon (LOC) fractions and enzyme activities following marshland reclamation and restoration may help us to

understand the responses of marshland ecosystems to land use changing.

The labile pool of soil organic matter could be used as a sensitive indicator of short-term changes in soil management. In soils, DOC, MBC and easily degradable organic carbon (EOC) have been identified as the important fractions in the active soil organic carbon pool. Of these fractions, DOC is probably the most bioavailable for soil microorganisms (Marschner and Kalbitz 2003). Compared with the total amount of organic carbon, the MBC and EOC may be more useful and sensitive measures for suggesting changes in the soil organic matter status (Powlson and Jenkinson 1981; Chan and others 2001). Although soil LOC fractions only represent a small percentage of the total soil organic carbon (SOC), changes in their quality and quantity could indicate changes in the total SOC (Gong and others 2008). Additionally, the microbial quotient (MBC/SOC) which can be interpreted as substrate available and the portion of total soil C immobilized in microbial cells (Yang and others 2010), has been used as an indicator of future changes in organic matter status due to alterations in land use (Moscatelli and others 2007).

Enzyme activity plays an important role in the biochemical functioning of soils, which includes soil organic matter formation and degradation, nutrient cycling and decomposition of xenobiotics (Acosta-Martínez and others 2007). In addition, changes in enzyme activities have the potential to affect wetland functions (Freeman and others 1997). In contrast, changes in land use may be reflected in the activities of soil enzymes. Invertase, β-glucosidase, urease and acid phosphatase are important enzymes in the soil C, N and P cycles. The invertase and β-glucosidase are related to the transformation and decomposition of SOC by hydrolyzing carbohydrates to sugar monomers and oligomers that are suitable for uptake by plants and microorganisms (Wan and others 2008; Sotomayor-Ramírez and others 2009). The urease plays a prominent role in nitrogen metabolism in nature by hydrolyzing urea to CO_2 and NH_3 (Klose and Tabatabai 2000). The acid phosphatase is a good indicator of organic P mineralization (Sinsabaugh 1994). Land use changes have significantly affected enzyme levels and activities in pastures and forests (Sicardi and others 2004; Acosta-Martínez and others 2007; Zornoza and others 2009). However, the impact of land use changes on the soil enzyme activities in marshlands has received less attention.

In the freshwater marsh region of the Sanjiang plain (in northeast China), the influence of marshland reclamation and restoration on LOC fractions and soil enzyme activities remains unclear. Therefore, this study was performed to investigate the changes in the DOC, EOC and MBC concentrations and the microbial quotient following marshland reclamation and restoration. In addition, we investigated the changes in invertase, β-glucosidase, urease and acid phosphatase activities following marshland reclamation and restoration. The objectives of this study were to determine sensitive indicators for detecting changes in soil quality and to evaluate the impacts of changing land use on marshland soil biochemical function.

Materials and Methods

Site Characteristics and Sampling Methods

The research site is located at the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences, Tongjiang City, Heilongjiang Province, China at approximately 47°35′N, 133°31′E. The mean annual precipitation is 500–650 mm (mainly in July and August), and the monthly mean temperature ranges from −20 °C in January to 22 °C in July. The altitude is approximately 56 m above sea level. Five adjacent sites were selected within a radius of 2 km. One site, the cultivated marshland, has been a soybean field for the last 20 years but was previously dominated by *Calamagrostis angustifolia*. Three of the sites were restored *C. angustifolia* marshlands that were abandoned 3, 6, or 12 years after they were cultivated for approximately 10 years. The fifth site was a natural *C. angustifolia* marshland that was selected as a reference. These five study sites were randomly assigned, and three 40 × 40 m^2 plots were randomly established in each field. The soil parent material was sediment originating from the Quaternary period, and the soils were classified as Albaquic Paleudalfs with a silty clay texture. Soil samples were collected in July 2009 from the upper soil horizon (0–20 cm) with a soil core sampler following the removal of the surface litter. In all cases, six soil cores were randomly collected. Soil samples were mixed and homogenized. A subsample was sieved (<2 mm) and kept at 4 °C before analyzing for DOC and MBC concentrations and soil enzyme activities. The bulk sample was air-dried and sieved prior to SOC and EOC determination. Biological and biochemical analyses were conducted within 2 weeks of soil collection.

Chemical, Biochemical and Biological Analyses

Soil DOC was determined following the procedures presented in Ghani and others (2003) and is briefly described here. Thirty milliliters of distilled water were added to soil samples prior to shaking for 30 min at approximately 30 rpm in an end-over-end shaker. Next, the samples were centrifuged for 20 min at 3,500 rpm. All supernatant solutions were filtered through 0.45-μm filters into separate vials prior to total C and inorganic C analysis. Soil DOC

was calculated by taking the difference between the total dissolved C and the dissolved inorganic C.

Soil EOC was determined by the method described by Blair and others (1995). Soil samples containing approximately 15 mg of C were weighed into plastic screw-cap centrifuge tubes, and 25 mL of a 333 mM $KMnO_4$ solution were added to each tube. Blank samples (containing no soil) were also analyzed during each run. The centrifuge tubes were tightly sealed and tumbled for 1 h at 12 rpm on a 15-cm-radius tumbler. Next, the tubes were centrifuged for 5 min at 2,000 rpm, and the supernatant solutions were diluted by 1:250 with deionized water. The absorbance of the diluted samples and standards at 565 nm was measured using a UV–Vis spectrophotometer (UV-7504, CANY, China). The change in $KMnO_4$ concentration was used to estimate the amount of oxidized C (assuming that 1 mM of MnO_4 was consumed in the oxidation of 9 mg of C). These results were expressed as mg g^{-1} soil.

Soil MBC was measured using the fumigation–extraction method (Lu 2000). Fumigated and non-fumigated moist soils were extracted by shaking for 30 min in 0.5 M K_2SO_4. Organic C in the extracts was analyzed by the dry combustion method using a Multi N/C 2100 analyzer (Analytik Jena AG, Germany). MBC was calculated using the following equation: MBC = E_C/0.45, where E_C is the difference in organic C between the fumigated and non-fumigated samples (Wu and others 1990).

SOC concentrations were determined with a Multi N/C 2100 analyzer (Analytik Jena AG, Germany) with the high temperature combustion method. In addition, the microbial quotient was calculated by dividing MBC by SOC. The field capacity of the soil was determined with the Wilcox method.

Soil β-glucosidase activities were measured with the method of Eivazi and Tabatabai (1988), using the substrate analogue *para*-nitrophenyl-β-D-glucopyranoside and were expressed as μg PNP g^{-1} h^{-1}. The urease and invertase activities in soil samples were determined following the procedures presented in Guan (1986), which uses urea and sucrose substrates, respectively. These measurements were expressed as mg $N–NH_4^+$ g^{-1} d^{-1} and mg glucose g^{-1} d^{-1}, respectively. The acid phosphatase activities were expressed as mg PNP g^{-1} 12 h^{-1} and were determined using *p*-nitrophenyl phosphate as a substrate (Zhao and Jiang 1986).

The aboveground biomass at the sampling site was determined prior to collecting soil samples. The plant material was clipped above the soil surface and oven-dried at 80 °C until a constant dry weight was obtained. The above-ground biomass was calculated with the followed equation: aboveground biomass = B/0.25 m^2, where B is the biomass obtained from the 50 × 50 cm^2 area.

Statistical Analysis

Data were analyzed using SPSS (v. 13.0) with an accepted significance level of $\alpha = 0.05$. An one-way analysis of variance (ANOVA) was performed to determine the significant differences in soil properties between the treatments. A separation of means was performed using the Fisher's least significant difference test. All data were normally distributed and met the assumptions of the ANOVA (data not shown). In addition, the Pearson's correlation coefficients between LOC fractions and soil enzyme activities were calculated.

Results

Soil and Vegetation Characteristics

Characteristics of soil and vegetation varied between the natural marshland, the cultivated marshland and the restored marshland (Table 1). The SOC concentration was significantly lower in the cultivated marshland than in the natural marshland (from 129.74 to 33.41 mg g^{-1}). In addition, the SOC increased to 59.06, 75.53 and 72.91 mg g^{-1} following 3, 6 and 12 years of restoration in the marshlands, respectively. The field capacity corresponded with the soil history as follows: natural *C. angustifolia* marshland > restored marshland > cultivated marshland. The soil pH ranged from 5.33 to 5.59 and was not significantly different among the restored marshlands, the cultivated marshland and the natural *C. angustifolia* marshland. In the marshland that was restored for 3 years, the vegetation consisted of *C. angustifolia* associated with *Vicia cracca*. Six years after restoration, *C. angustifolia* recovered completely. During the restoration, plant height increased significantly. In the marshland that was restored for 12 years, vegetation had the greatest height and aboveground biomass at 93.89 cm and 532.76 g m^{-2}, respectively.

DOC, EOC, MBC and Microbial Quotient

DOC concentrations were the highest in the marshland soils (218.43 μg g^{-1}) and decreased following cultivation in the cropland soils (36.65 μg g^{-1}) (Fig. 1A). Following marshland restoration for 3, 6 and 12 years, the DOC concentrations increased to 146.42, 105.34 and 149.74 μg g^{-1}, respectively, relative to the DOC concentrations in the cultivated marshland soils.

In contrast, the EOC concentrations were lowest in the natural marshland soils (13.05 mg g^{-1}) and increased by 45.36 % following cultivation (Fig. 1B). Furthermore, the EOC concentrations in the 3- and 6-year restored

Table 1 Soil organic C, field capacity, soil pH, vegetation type, vegetation height, and aboveground biomass in the cultivated marshland, the restored marshland and the natural marshland

Restoration time	Soil characteristics			Vegetation characteristics		
	Soil organic C (mg g^{-1})	Field capacity (%)	Soil pH (H_2O)	Vegetation type	Vegetation height (cm)	Above-ground biomass (g m^{-2})
0 y	33.41 ± 1.45[d]	71.71 ± 2.49[c]	5.36 ± 0.03[a]	*Glycine max* Merr	71.10 ± 2.09[c]	454.73 ± 12.55[b]
3 y	59.06 ± 0.91[c]	89.28 ± 1.31[b]	5.59 ± 0.19[a]	*Calamagrostis angustifolia*. associated *Vicia cracca*	79.33 ± 5.86[b]	353.07 ± 52.54[d]
6 y	75.53 ± 2.07[b]	87.51 ± 1.06[b]	5.50 ± 0.09[a]	*Calamagrostis angustifolia*.	82.83 ± 5.02[b]	383.64 ± 24.56[c,d]
12 y	72.91 ± 1.59[b]	88.10 ± 0.07[b]	5.56 ± 0.05[a]	*Calamagrostis angustifolia*.	93.89 ± 3.60[a]	532.76 ± 37.79[a]
NM	129.74 ± 4.27[a]	94.50 ± 1.66[a]	5.33 ± 0.04[a]	*Calamagrostis angustifolia*. associated *Salix brachypoda*	86.79 ± 2.96[a,b]	420.24 ± 64.70[b,c]

The soil samples obtained from the 0-, 3-, 6- and 12-year restored marshlands are represented by 0, 3, 6 and 12 year, respectively, and the natural *C. angustifolia* marshland is denoted as NM. The mean values are represented by the notation ±SE ($n = 3$). The means with different lowercase letters in the same column are significantly different ($P < 0.05$)

marshland soils were higher than in the natural marshland soils. No significant differences were observed between the natural marshland and the 12-year restored marshland.

The largest MBC concentration was in the natural marshland soils (4314.51 μg g^{-1}), which was 8.4 times greater than the MBC in the cultivated marshland soils (513.45 μg g^{-1}) (Fig. 1C). The soil MBC concentration gradually increased with time (restored for 12 years > restored for 6 year > restored for 3 years > cultivated marshland). However, the MBC concentrations at the 12-year restoration site never reached those found in the natural marshland.

The microbial quotient was significantly different between the natural marshland, the cultivated marshland and the restored marshland (Fig. 1D). Marshland cultivation caused a significant decline in the microbial quotient (from 3.33 to 1.54 %). In addition, following 3, 6 and 12 years of cultivated marshland restoration, the microbial quotient significantly increased to 2.71, 2.41 and 2.75 %, respectively.

Soil Invertase, β-Glucosidase, Urease and Acid Phosphatase Activities

The cultivated marshland soils had the lowest invertase, β-glucosidase and urease activities (Fig. 2). The restoration of marshland resulted in an increase in the invertase and β-glucosidase activities (Fig. 2A, B). The invertase activities corresponded with soil history as follows: natural marshland > restored for 12 years > restored for 6 year = restored for 3 years > cultivated marshland (Fig. 2A). The β-glucosidase activities in the natural marshland soils (250.01 μg PNP g^{-1} h^{-1}) were significantly higher than in the restored and cultivated marshlands (Fig. 2B). The soil urease activities in the cultivated marshland soils were lower than in the other soils but recovered after the cultivated marshland restoration (Fig. 2C). The urease activity in the 3 year restored marshland (0.93 mg N–NH_4^+ g^{-1} d^{-1}) was three times higher than in the cultivated marshland (0.31 mg N–NH_4^+ g^{-1} d^{-1}). In addition, there were no significant differences between the urease activities in the 12-year restored marshland and the natural marshland. Soils from the 6-year restored marshland had the highest acid phosphatase activities (2.09 mg PNP g^{-1} 12 h^{-1}). There were no significant differences in the acid phosphatase activities between the cultivated and natural marshland soils (Fig. 2D).

Relationships Between LOC Fractions and Soil Enzyme Activities

A correlation analysis showed that the DOC concentrations were positively correlated with the MBC and SOC concentrations ($r = 0.910$ and 0.885, $P < 0.01$, $n = 15$, respectively) and the invertase, β-glucosidase and urease activities ($r = 0.925$, 0.916 and 0.810, $P < 0.01$, $n = 15$, respectively) (Table 2). In addition, Table 2 shows the significant positive correlations between the MBC concentrations and the invertase, β-glucosidase and urease activities ($r = 0.974$, 0.923 and 0.663, $P < 0.01$, $n = 15$, respectively). Positive correlations between the microbial quotient and the invertase and β-glucosidase activities were also observed ($r = 0.959$, 0.787 and 0.687, $P < 0.01$, $n = 15$, respectively). Furthermore, the microbial quotient was correlated with the DOC and MBC concentrations ($r = 0.898$ and 0.908, $P < 0.01$, $n = 15$, respectively). Positive correlations were also observed between the invertase, β-glucosidase and urease activities. Such relationships show co-action in the mineralization of soil organic matter. However, no relationships were found between the acid phosphatase activities and the LOC fractions.

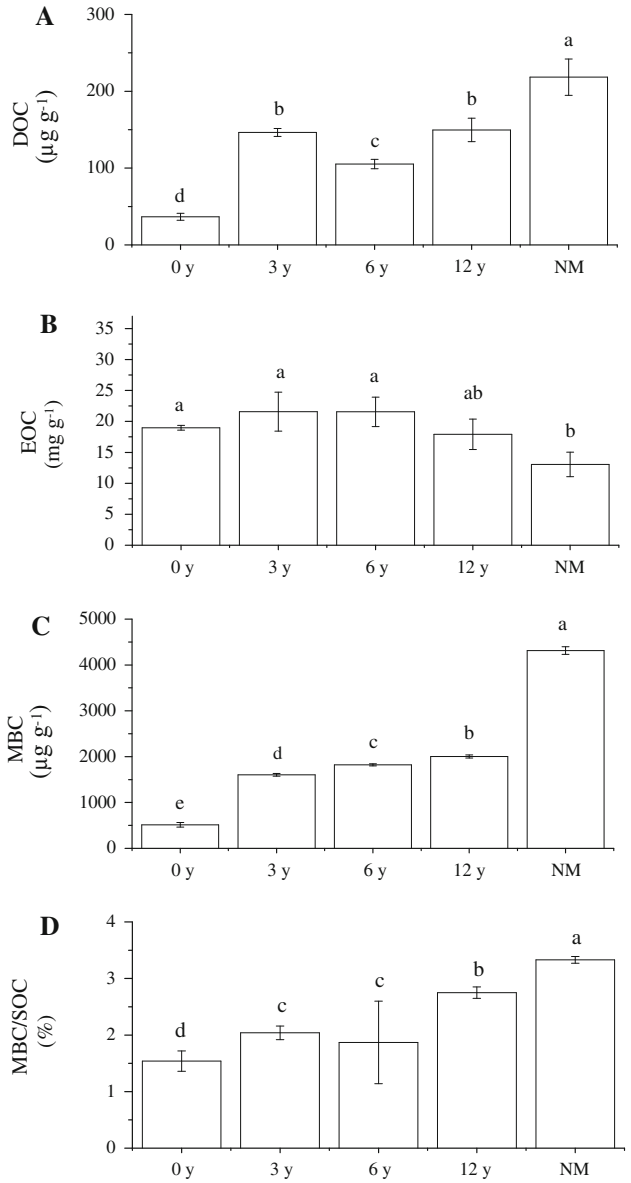

Fig. 1 DOC (**A**), EOC (**B**), MBC (**C**) and microbial quotient (MBC/SOC) (**D**) in the cultivated, restored and natural marshland soils. Samples obtained from 0, 3, 6 and 12 years of restored marshland are represented by 0, 3, 6 and 12 y, respectively, and NM represents the natural *C. angustifolia* marshland. The mean values with standard errors (represented by *bars*) ($n = 3$) are presented. The means with *different lowercase letters* are significantly different at $P < 0.05$

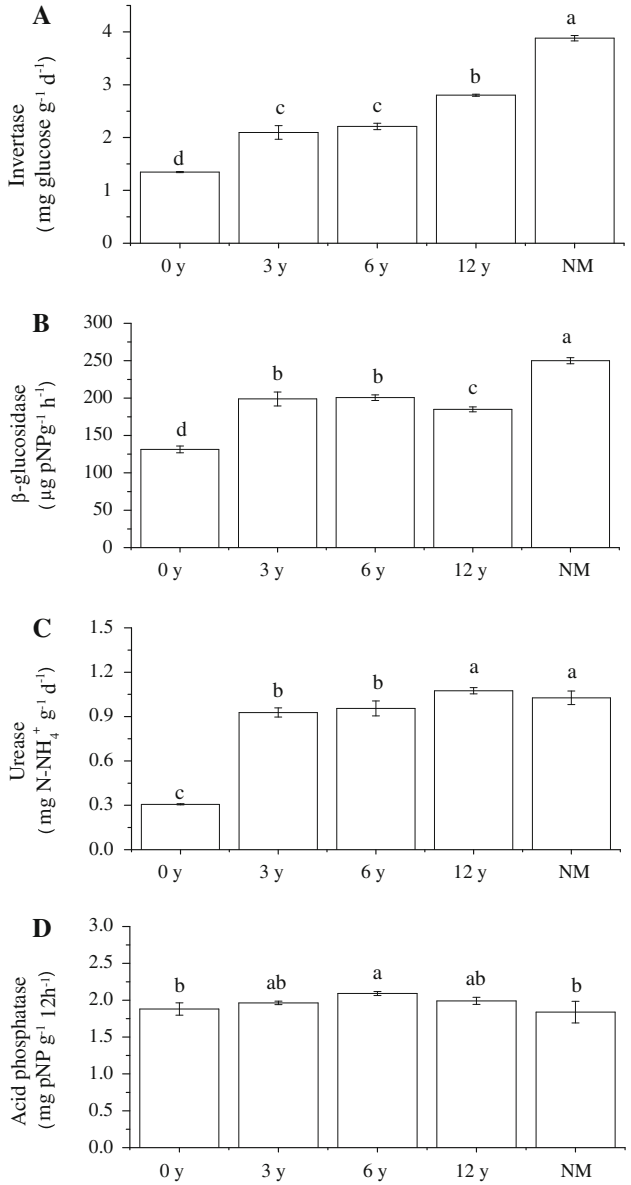

Fig. 2 Soil invertase (**A**), *β*-glucosidase (**B**), urease (**C**) and acid phosphatase (**D**) activities in the cultivated, restored and natural marshland soils. Samples obtained from 0, 3, 6 and 12 years of restored marshland are represented by 0, 3, 6 and 12 y, respectively, and NM represents the natural *C. angustifolia* marshland. The mean values with standard errors (represented by *bars*) ($n = 3$) are presented. The means with *different lowercase letters* are significantly different at $P < 0.05$

Discussion

Compared with the natural *C. angustifolia* marshland, 74 % of the soil C was lost following the cultivation of marshland for 20 years. These results support previous findings that C loss 78 % following 15 years of marshland cultivation in the Sanjiang Plain (Zhang 2006). This loss in SOC probably resulted from reduced input of organic matter (organic materials were removed every autumn), reduced physical protection of SOC (Davidson and Ackerman 1993) and increased soil C mineralization as a result of tillage (Raiesi 2006; Zhang and others 2007a). Marshland restoration promoted the accumulation of SOC by enhancing the residue and the physical protection provided by the vegetation. However, for 12 years, the SOC concentrations did not reach the concentrations found in the natural marshland. Similarly, Cui and others (2009) found

Table 2 Correlation coefficients between LOC fractions and soil enzyme activities (n = 15)

	Invertase	β-glucosidase	Urease	Acid phosphatase	DOC	EOC	MBC	MBC/SOC	SOC
Invertase	1.000								
β-glucosidase	0.880**	1.000							
Urease	0.731**	0.799**	1.000						
Acid phosphatase	−0.235	−0.040	0.278	1.000					
DOC	0.925**	0.916**	0.810**	−0.168	1.000				
EOC	−0.687**	−0.416	−0.162	0.453	−0.543*	1.000			
MBC	0.974**	0.923**	0.663**	−0.273	0.910**	−0.680**	1.000		
MBC/SOC	0.959**	0.787**	0.687**	−0.257	0.898**	−0.707**	0.908**	1.000	
SOC	0.965**	0.927**	0.686**	−0.219	0.885**	−0.634*	0.991**	0.875**	1.000

*, ** indicate significance at $P < 0.05$ and <0.01, respectively

that SOC increased continuously during 7 years of wetland restoration in the Yellow River Delta, China.

Compared with the natural marshlands, cultivation led to an 83 % loss in DOC. This finding was consistent with that of Gregorich and others (2000), who reported that DOC concentrations were lower under continuous cropping systems than under grass systems. The primary sources of DOC include exudates from living vegetation, incompletely decomposed organic matter and vegetation litter (Paterson and others 1997; Guo and others 2010). DOC in cropland soil declines due to the reduction of photosynthate. In addition, the grass root layers were damaged following yearly wetland cultivation and harvest, which resulted in a sharp reduction in the quantity of plant matter returned to the soil (Zhang 2010). After abandonment of the cultivated marshland, the observed increase in soil DOC probably resulted from reduced soil disturbance, increased vegetation litter and increased SOC. Consisted with our findings, Jones and others (1998) found that the subsurface layer fixes carbon, which increased the DOC concentrations in a grassland model ecosystem. In addition, increasing amounts of root exudates from vegetation were an important source of DOC (Kang and others 2001).

Cultivation resulted in higher EOC concentrations than in the natural marshland. The EOC concentration is an estimate of active carbon and measures simple carbohydrates, amino acids and sugars in SOC that are easily hydrolyzed and oxidized (Loginow and others 1987). As a result, EOC concentrations are significantly influenced by nutrients and water. Fertilizer applications in cropping systems are responsible for increasing soil LOC (Contech and others 1997). Diammonium phosphate and urea were used as fertilizer in the cultivated marshland. Yang and others (2005) reported that the application of fertilizers significantly increased the EOC pool. The EOC concentration was also impacted by soil moisture. During alternating periods of wetting and drying, the soil EOC was higher than when subjected to continuous saturation (Yang and others 2005). Therefore, exposing the soil to fertilizers and the drought conditions that are used in farming practices increased the EOC pool in the cultivated marshland.

The soil MBC pool decreased as a result of marshland reclamation, which indicated that marsh cultivation reduced the amount of carbon immobilized as microbial biomass. During marshland restoration, the MBC pool gradually increased over time. This change was consistent with the change reported by Zhang and others (2007a, b) in Sanjiang wetland soils. Due to the reduced soil disturbance and the increased litter input, marshland restoration facilitates the development of microorganisms in soils. A positive correlation between the MBC and SOC concentrations was observed. Thus, higher levels of soil organic matter may favor the growth of microbial populations and result in greater microbial biomass (Jia and others 2010).

The microbial quotient (MBC/SOC), which can be interpreted as substrate available and the portion of total soil C immobilized in microbial cells, is a sensitive indicator of both organic C and microbial biomass (Garcia and others 2002). Larger ratios imply an increase in the availability of fresh substrates, while smaller ratios imply a reduction in the availability of substrates (Anderson and Domsch 1986). Jenkinson and Ladd (1981) reported that MBC typically comprises 1–5 % of SOC. Similarly, in this study, the microbial quotient varied from 1.54 to 3.33 %. The natural marshland soils had the highest microbial quotient, which indicated that a large proportion of the microbial biomass was supported by high C substrate availability. The cultivated marshland soils had the lowest microbial quotient. The results from Zhang and others (2007a) showed that cultivation practices have a negative influence on the microbial quotient. Our study showed a significant increase in microbial quotient after restoration

of cultivated marshland. This finding suggests that the fraction of organic substrate that is available for soil microorganism increases following marshland restoration.

Nutrient cycling in soils involves biochemical, chemical and physicochemical reactions. Biochemical reactions are catalyzed by enzymes (Salazar and others 2011). A suite of soil enzymes operate cooperatively for the decomposition of organic matter. Each enzyme has its own substrate and ability to catalyze specific biochemical reactions. Marshland cultivation decreased the invertase, β-glucosidase and urease activities. This decrease in activity will reduce C and N cycling between the soils organic matter pools. These results provide insight into how soil C and N cycling are affected by land use changes in marshlands. The intermediate status of the enzyme activities in the marshlands from which cultivation was abandoned suggests that the biochemical functions of the soil ecosystem are being restored by providing available substrates for the enzymes. A large amount of litter was returned to the soil after abandonment of the cultivated marshland. The decomposition of plant matter is accompanied by the stimulation of a succession of soil enzymes, which include invertase (Wang and others 2010) and β-glucosidase (Steffen and others 2007). Both of these enzymes degrade the extractive component of the litter containing compounds (Moorhead and Sinsabaugh 2000). In addition, the changes in soil C-transforming enzyme activities following the marsh land use changes may result from the variations in the DOC and MBC pools. Soil invertase and β-glucosidase activities were positively correlated with DOC concentrations. This positive relationship was also reported by Freeman and others (1998) and Cang and others (2009). These relationships probably occurred because DOC contains both substrates and end products of enzymic reactions of varying molecular weight (Bonnett and others 2006). Increased DOC in soils results in increased substrate abundance for microbial metabolism and supports the synthesis of new enzymes (Freeman and others 1998; Kang and others 2005). Invertase and β-glucosidase probably originated from microorganisms. This hypothesis is supported by the significant correlations between the invertase and β-glucosidase activities and the MBC concentrations. Similar significant correlations have been identified in other studies (Turner and others 2002; Wan and others 2008). These results support the relationship between the microbes and the invertase and β-glucosidase (both of which take part in the recycling of C) and confirms the potential of these enzymes for indicating microbial activity. In addition, Tripathi and others (2007) also considered that microbial biomass is an important labile fraction of soil organic matter and a potential source of enzymes in soils.

Marshland cultivation decreased the urease activity, which indicated that the reduced metabolism in the cultivated soils affected the biological transformation of N. Burket and Dick (1998) reported that inorganic N fertilizers in the form of NH_4^+ could suppress the urease activity. This suppression of the urease may be explained by the large number of end products containing the target nutrient that inhibit the microbial induction of urease (Tscherko and others 2003). Urease activities increased following marshland restoration and reached the natural marshland levels following 12 years. In contrast, marshland cultivation and restoration had no significant impact on the acid phosphatase activity, mainly because soil pH did not change with changing land use (Table 1). The phosphatase are crucial in the transformation of organic P. In addition, phosphatase are significantly affected by soil pH, which controls P availability independently of organic matter concentrations or levels of disturbance (Acosta-Martínez and others 2008). Additional research is required to determine the relationships between the acid phosphatase activity and the P mineralization in marshland soils.

Our study has shown that land use has a significant impact on soil LOC fractions and enzyme activities that are involved in C and N cycling in marshlands. Based on these results, we conclude that understanding the sensitivity of indicators to marshland cultivation and restoration may help us to understand the soil ecosystem responses to changing land use in marshlands, especially with regard to soil biogeochemical processes. To achieve this objective, the present data of the soil characteristics in only one undisturbed, one cultivated and three different ages of restored marshland are limited, furthermore, considerable the differences that exist between the short-term and long-term effects, the multiple sampling and the long-term studies of soil characteristics in this region need further elucidation.

Conclusions

Our results demonstrated that marshland cultivation leads to decreases in the DOC, MBC and SOC concentrations, the microbial quotient, and the invertase, β-glucosidase and urease activities. However, marshland cultivation resulted in a higher EOC pool. Restoration of cultivated marshland improved soil quality and marshland biochemical function by increasing the DOC, SOC, and MBC concentrations, the microbial quotient, and the invertase, β-glucosidase and urease activities. These results indicate that land use in the marshland had a strong impact on soil function and composition. In addition, these results confirmed that DOC, MBC, microbial quotient, and the invertase, β-glucosidase and urease activities are potential indicators for soil quality and environmental impact in marshlands. Considering the significant impacts of marsh land use on soil chemical and

biochemical properties, the conservation of marshland in the future should be emphasized. In addition, the government should continue to implement policies for returning farmland to marshland in areas that are not suitable for cultivation.

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